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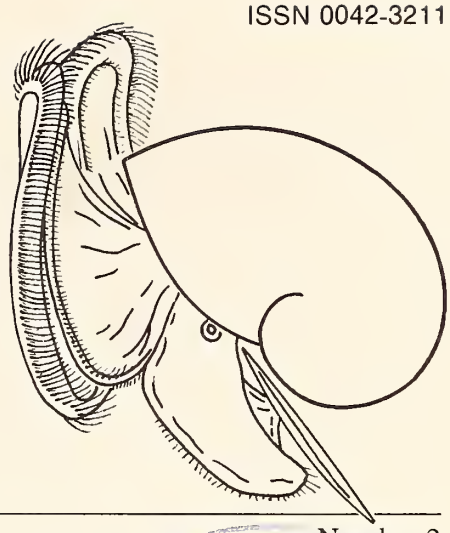
THE VELIGER



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THE VELIGER

Allozyme Variation in the Australian Camaenid Land Snail *Cristilabrum primum*: A Prolegomenon for a Molecular Phylogeny of an Extraordinary Radiation in an Isolated Habitat

by

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Abstract. A group of 27 species of land snails belonging to three genera have been discovered isolated in the 52-km long limestone Ningbing Range in northwest Australia. These endemic species have very restricted geographic ranges (averaging 1 km^2) and are typically allopatric. As the three genera have no known close relatives, it is assumed that the taxa evolved *in situ* since camaenids colonized Australia in the Miocene. We studied genetic variation in one species and compared it with two congeners and representatives of the other two genera to provide a basis for the development of a molecular phylogeny of this remarkable radiation. Electrophoretically detected variation at 21 allozyme loci in *Cristilabrum primum* is described; this species is shown to be highly variable ($P = 0.71$, $\bar{H} = 0.22$) and outcrossing. Multilocus differentiation within *C. primum* is small (Nei's genetic distance, $D = 0$–0.02) and typical of conspecific populations. Preliminary data show *C. primum* is weakly differentiated from one parapatric neighbor (*C. grossum*: $D = 0.04$) and well-differentiated from the other (*C. monodon*: $D = 0.17$); all three species are, however, well differentiated anatomically and conchologically. The genetic distances between *Cristilabrum* and the other two genera (*Turgenitubulus*, $\bar{D} = 0.27$; *Ningbingia*, $\bar{D} = 0.50$) are larger and bode well for the development of an allozyme-based phylogeny.

INTRODUCTION

An extraordinary radiation of camaenid land snails has been discovered in an isolated series of limestone hills in the northeast corner of Western Australia. Twenty-nine endemic species of pulmonates have been described from a narrow 52-km long chain of isolated limestone hillocks, the Ningbing Range and adjacent Jeremiah Hills (SOLEM, 1981, 1985, 1988a, b, 1989a, b). These species are mainly allopatric in distribution and the 27 species of camaenids are remarkable for their very restricted geographic ranges, typically less than 1 km^2. They occupy all available habitat in these hills, the low remnants of a Devonian reef, but cannot live on the surrounding alluvial plain and are not found in nearby sandstone ranges. The various species are well characterized on the basis of anatomical and conchological features and all but one are placed in three restricted endemic genera. The exception, *Ordtrachia elegans* Solem, 1988, belongs to a distantly related East Kimberley genus. The other three genera have not been found elsewhere, and it appears that their 26 species evolved *in*

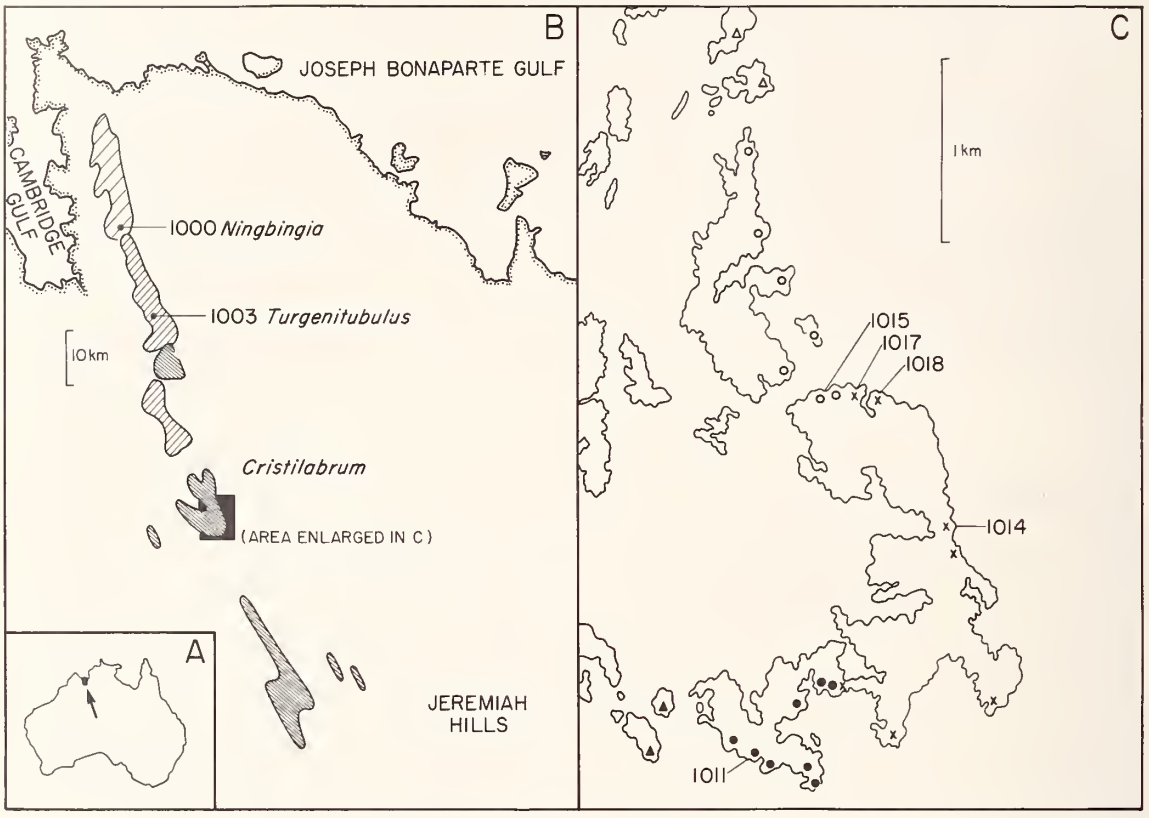

Figure 1

Locality map. A. Position of the Ningbing Range. B. Diagram showing the geographic distribution of *Ningbingia, Turgenitubulus,* and *Cristilabrum* in the Ningbing Range and Jeremiah Hills. Collection sites of numbered samples and generic ranges are shown. C. Enlargement of the central portion of the range of *Cristilabrum* showing position of samples relative to the main limestone outcrops: *C. primum* (×), *C. monodon* (○), *C. grossum* (●), other *C.* sp. (△▲). Source: SOLEM, 1988b.

situ since camaenids first gained access to Australia from Asia during the Miocene (SOLEM, 1988a, b). The Ningbing radiation occurs in "a little world within itself," to recall DARWIN's (1839:454) phrase, and its phylogenetic analysis begs attention and comparison with other better known examples of evolution in isolated microcosms. In this paper, we describe the genetic variation in one species as a prolegomenon to preparation of a molecular phylogeny of the radiation as a whole.

The Ningbing Range and Jeremiah Hills are located on the east fringe of the Kimberley Region, 50–100 km north of Kununurra, Western Australia (Figure 1A). The three endemic camaenid genera are allopatric, with *Ningbingia* in the North Ningbing Range, *Turgenitubulus* in the Central Ningbing Range and adjacent hills, and *Cristilabrum* in the south portion of the Central Ningbing Range, South Ningbing Range, and adjacent Jeremiah Hills (Figure 1B). With many isolated hills yet to be explored, 27 endemic species are now recognized: *Ningbingia* Solem, 1981, with six species and one subspecies; *Turgenitubulus* Solem, 1981, with eight species; and *Cristilabrum* Solem, 1981, with 13 species (12 described). The species' geographic ranges have been described by SOLEM (1988b); they are all continuous and discrete rather than interspersed. The median areas for species geographic ranges within genera are *Ningbingia* 1.05 km^2, *Turgenitubulus* 1.66 km^2, and *Cristilabrum* 0.63 km^2. There are seven cases of "same rock" microsympatry involving two species (and one involving three species). Sympatric taxa are typically characterized by marked differences in the terminal genitalia and easily observed shell differences. Undoubtedly, additional species remain to be discovered.

The Ningbing radiation occurs in a remote area of difficult access. Field work is impossible during the wet season associated with the monsoon (November through March). Some biological observations have been made early (May–June) and at the end of the dry season (November) when the snails are found estivating (SOLEM & CHRISTENSEN, 1984). All the endemic camaenids are "free sealers" and are found lying loose in limestone rubble with a sheet of calcified mucus closing the shell aperture. During the 7–8 month dry season they are inactive and depend on stored

food for survival. The snails first function as males at the beginning of their third wet season; in their fourth and subsequent wet seasons they are outcrossing hermaphrodites. Mating has not been observed, but in other probably related camaenids it is reciprocal. Longevity is unknown, but observations of other Kimberley camaenids suggest 10–15 yr is not unreasonable. The snails are highly clumped in their dispersion in the dry season; SOLEM (1988b) discusses the highly fragmented pattern of dry season distribution and the extremely small volumes (<1 m^3) occupied by some successful colonies. Although quantitative data are lacking, we get the impression that many estivation sites shelter closer to 100 than 1000 snails. A metapopulation model, made up of many small to very small subpopulations, seems appropriate to today's Ningbing endemics.

In the absence of a fossil record, the phylogeny of the Ningbing radiation must be deduced from a study of the living forms. Four independent data sets (based on shell morphology, genital anatomy, biogeography, and allozymes) provide excellent data for the preparation of hypothetical phylogenies. Observations of feeding, excretory, muscle, digestive, and nervous systems showed uniform patterns and thus could not be utilized. In order to set the stage for the subsequent development of an allozyme-based phylogeny, we measured intraspecific variation in one species, *Cristilabrum primum* Solem (1981), which is common within a restricted area (1.2 km^2) in the South Ningbing Ranges (SOLEM, 1988b). It occupies the central part of a 3.5-km long hill and is flanked to the northwest and southeast by *C. monodon* Solem (1985) and *C. grossum* Solem (1981), respectively (Figure 1C). These three species are the only members of this radiation on this particular hill and their ranges are allopatric and presently separated from one another by 100-m wide areas lacking suitable habitats. Consistent shell and anatomical differences between these three species have been described elsewhere (SOLEM, 1981, 1985) and are summarized in Table 4. To test the resolving power of an allozyme-based phylogeny, we compared *C. primum* with its two congeneric neighbors and to one representative of each of the other two endemic genera: *Ningbingia australis australis* Solem (1981), and *Turgenitubulus tanmurranus* Solem (1985). This is the first report of genetic variation in the Australian camaenid land snails so the choice of *C. primum* seems appropriate.

Table 1

Presumptive loci and electrophoretic conditions for camaenids

Allozyme	Gene symbol	E.C. No.	Buffer*
Esterase 1	*Es-1*	3.1.1.1	TC 6.0
Glucose-6-phosphate dehydrogenase	*G6pd*	1.1.1.49	TBE 9/8
Glucose phosphate isomerase	*Gpi*	5.3.1.9	TC 6.0
Glutamic-oxaloacetic transaminase soluble	*Got-1*	2.6.1.1	TC 6.8
Glutamic-oxaloacetic transaminase mitochondrial	*Got-2*	2.6.1.1	TC 6.8
Glyceraldehyde-3-phosphate dehydrogenase	*Gapd*	1.2.1.12	TC 6.8
Glycerol-3-phosphate dehydrogenase	*Gpd*	1.1.1.8	TBE 9/8
Isocitrate dehydrogenase, soluble	*Idh-1*	1.1.1.42	TC 6.8
Isocitrate dehydrogenase, mitochondrial	*Idh-2*	1.1.1.42	TC 6.8
Lactate dehydrogenase	*Ldh*	1.1.1.27	TBE 9/8
Malate dehydrogenase, soluble	*Mdh-1*	1.1.1.37	TC 6.0
Malate dehydrogenase, mitochondrial	*Mdh-2*	1.1.1.37	TC 6.0
Malic enzyme cytoplasmic	*Me*	1.1.1.40	TBE 9/8
Mannose phosphate isomerase	*Mpi*	5.3.1.8	TC 6.0
Peptidase 1 (leucineamino)	*Pep-1*	3.4.11/13	TBE 9/8
Peptidase 2 (L-leucyl-L-alanine)	*Pep-2*	3.4.11/13	TBE 9/8
Peptidase 3 (leucyl glycyl glycine)	*Pep-3*	3.4.11/13	TBE 9/8
Phosphoglucomutase 1	*Pgm-1*	2.7.5.1	TBE 9/8
Phosphoglucomutase 2	*Pgm-2*	2.7.5.1	TBE 9/8
Phosphogluconate dehydrogenase	*Pgd*	1.1.1.43	TC 6.8
Sorbitol dehydrogenase	*Sord*	1.1.1.14	TBE 9/8

* *TBE 9/8.* 0.087 M Tris, 0.086 M borate, 0.001 M EDTA, pH 9.0; diluted 1:3 for gels. 0.5 M Tris, 0.065 M borate, 0.02 M EDTA, pH 8.0; undiluted for electrodes (17 hr 50 V).

TC 6.0. 0.378 M Tris, 0.165 M citrate, pH 6.0; 13.5 ml diluted to 400 ml for gel and undiluted for electrodes (18 hr 70 V).

TC 6.8. 0.188 M Tris, 0.065 M citrate, pH 6.8; diluted 1:9 for gels and 1:5 for electrodes (18 hr 50 V).

MATERIALS AND METHODS

All snails were collected in late May 1984 by Alan Solem, Field Associate Laurie Price, or a student assistant, K. C. Emberton. At that time of year, the snails were two months into the 6–8 month dry season and all were found estivating. Typically, the sample was collected from about 1 m^3 of rock rubble or from crevices in an area of less than 3 m^2 of limestone. Live individuals of *Cristilabrum primum* were collected at three stations, WA-1017 and WA-1018 at the north end and WA-1014 in the middle of the species range (Figure 1C). These and other collection stations are mapped and described by SOLEM (1981, 1988b). *Cristilabrum primum* has a total linear range of about 1.2 km; stations WA-1017 and WA-1018 are about 50 m apart across a gully and about 0.9 km north of WA-1014. *Cristilabrum monodon* occurs immediately northwest of *C. primum* and was sampled at WA-1015, only 200 m west of WA-1017. *Cristilabrum grossum* replaces *C. primum* to the southwest on the same rock mass and was sampled at WA-1011 about 1.6 km southwest of WA-1014. The eight

species of the genus *Turgenitubulus* replace *Cristilabrum* to the north; we describe here the variation in *T. tanmurranus* from about 18 km north of *C. primum* at WA-1003. Further north again are six species of *Ningbingia* represented here by *N. australis* from WA-1000, about 31 km north of *C. primum*.

Tissues for allozyme analysis were prepared on the day of collection. Snails were quickly activated by short exposure to wet paper towels and the posterior portion of the cephalopodal mass was amputated with a razor and stored individually in cryogenic vials in liquid nitrogen. For electrophoresis in 1986–1987, the individual tissue samples were thawed and quickly minced in 0.1 mL of cold grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NADP, pH 7.0) with mounted needles and a glass rod. Each homogenate was centrifuged at 10,000 g for 2 min and the supernatant was absorbed onto 3 × 9 mm paper wicks cut from Whatman No. 3 chromatography paper. The wicks were inserted into 12% horizontal gels made of Sigma starch (Sigma Chemical Co., St. Louis, MO). Electrophoretic conditions for the 21 allozymes reported here are described in Table 1. Four or five slices were cut from each gel after 17–18 hr of electrophoresis and each slice was stained for a specific enzyme following standard methods (Harris & Hopkinson, 1978; Richardson *et al.*, 1986). The esterase substrate was alpha-naphthyl acetate. Three peptidases were studied: leucine aminopeptidase, L-leucyl-L-alanine peptidase and leucyl-glycyl-glycine peptidase. Snails from different samples were run on each gel to facilitate comparisons and a bromophenol blue dye was used to track the front. Isozymes were numbered and allozymes were assigned superscript letters a, b, *etc.*, in order of decreasing anodal mobility. Relative mobilities of all electromorphs were determined by measurement and all gels were photographed immediately after patterns were resolved. Each allozyme is identified in Table 1 by its International Union for Pure and Applied Chemistry and International Union for Biochemistry Enzyme Commission (E.C.) number. Enzyme abbreviations (gene symbols) conform to human gene nomenclature (Roychoudhury & Nei, 1988) and are typeset in capital letters to indicate the protein and in lowercase italics to indicate the presumed allele.

Multilocus genotype data for all individuals were entered into a computer and a series of statistical analyses was performed with the BIOSYS-1 computer program (Swofford & Selander, 1981). For each sample the number of alleles per locus (A), the proportion of loci polymorphic (P), and the mean individual heterozygosity ($\bar{H}$) were determined. A locus was considered polymorphic if more than one allele was detected; $\bar{H}$ was determined by direct count. Genotype frequencies at each polymorphic loci were tested for their agreement with Hardy-Weinberg expectations for a panmictic population by χ^2-test (with Levene's [1949] correction for small sample size) where appropriate and by the Fisher exact test. Intra- and interpopulation genetic structuring was examined using Wright's (1978) hierarchical F-statistics (F_{is}, F_{it} and F_{st}) calculated for each locus and each sample. Intersample differentiation was estimated using the unbiased genetic distance coefficients (D) of Nei (1978) and the modified Rogers' distance coefficient (Wright, 1978). A phenogram was constructed using the UPGMA clustering algorithm (Sneath & Sokal, 1973) based on Nei's D. In addition, optimized unrooted and rooted trees were constructed by the Distance Wagner method based on the modified Rogers' distance.

RESULTS

Looking first at the large sample ($\bar{N} = 51$) of *Cristilabrum primum* from site WA-1018, we note that this population is highly variable at the 21 loci examined: 15 of the 18 loci found to be variable in the five species studied were polymorphic in *C. primum* ($\bar{P} = 0.71$). A similar high value was obtained for the variable proportion of each individual's genome, $\bar{H} = 0.22$. Comparably relatively high values for P and H were observed in the other two samples of this species; the slightly lower absolute values for samples WA-1017 and WA-1014 are attributable to smaller sample sizes (Table 2).

Relatively high levels of polymorphism and individual heterozygosity were also observed in the congeneric samples of *Cristilabrum monodon* and *C. grossum*. Excluding the small sample WA-1017, we note that for *Cristilabrum* $\bar{P} = 0.63$ (0.57–0.71) and $\bar{H} = 0.19$ (0.15–0.22). Slightly less variation was observed in *Turgenitubulus tanmurranus*: $P = 0.48$, $\bar{H} = 0.17$. In contrast, *Ningbingia australis* is only about one-third as variable: $P = 0.19$, $\bar{H} = 0.08$.

Thirty-three alleles were segregating at the 15 polymorphic loci detected in sample WA-1018 of *Cristilabrum primum*. In all 15 cases, chi-square tests of genotype frequencies provided no significant evidence for significant departure from random mating expectations ($p \geq 0.10$). The same conclusion was reached using the more appropriate Fisher exact probability tests, and with the two smaller conspecific samples of *C. primum* and the samples of *C. grossum* (12 tests) and *Ningbingia australis* (4 tests). Similarly, departures from panmictic expectations in 3 of 13 chi-square tests involving *C. monodon* and 1 of 10 tests involving *Turgenitubulus tanmurranus* proved insignificant when Fisher's exact test was applied. We conclude that these hermaphrodites have population structures involving random mating and insignificant inbreeding. This conclusion is supported by the observation of an overall $F_{is} = 0.049$, a value not significantly different from zero. In contrast, the overall $F_{st} = 0.45$ indicates significant intersample heterogeneity.

Two measures of intersample genetic divergence are shown in Table 3. Between the three samples of *Cristilabrum primum*, Nei's unbiased genetic distance was $\bar{D} = 0.010$ (range: 0.00–0.016). *Cristilabrum primum* was found

Table 2

Allele frequencies for 18 polymorphic loci in 7 samples of camaenids (*Ningbingia, Turgenitubulus, Cristilabrum*) from the Ningbing Range with summary statistics of genetic variability.*

Locus/ allele	*N. australis* 1000	*T. tanmurranus* 1003	*C. monodon* 1015	*C. primum* 1017	*C. primum* 1018	*C. primum* 1014	*C. grossum* 1011
Es-1							
n	16	12	13	5	51	10	16
a	—	—	0.54	0.80	0.38	0.60	0.16
b	1.00	0.12	0.35	0.20	0.62	0.40	0.84
c	—	0.71	0.11	—	—	—	—
d	—	0.17	—	—	—	—	—
Gpi							
n	14	12	13	5	56	11	16
a	—	0.08	—	0.20	0.07	0.05	—
b	1.00	0.63	1.00	0.60	0.75	0.77	0.69
c	—	0.29	—	0.20	0.18	0.18	0.31
Got-1							
n	16	12	13	5	54	11	16
a	—	1.00	0.88	1.00	1.00	1.00	1.00
b	—	—	0.12	—	—	—	—
c	1.00	—	—	—	—	—	—
Gpd							
n	13	12	13	5	44	8	13
a	—	1.00	—	—	—	—	—
b	1.00	—	0.15	1.00	1.00	0.88	1.00
c	—	—	0.85	—	—	0.12	—
Idh-1							
n	16	12	13	5	56	9	16
a	1.00	1.00	1.00	0.60	0.75	0.78	1.00
b	—	—	—	0.40	0.25	0.22	—
Idh-2							
n	13	12	13	3	39	9	12
a	1.00	1.00	1.00	0.67	0.76	0.83	0.79
b	—	—	—	0.33	0.24	0.17	0.21
Ldh							
n	16	12	13	5	26	10	13
a	1.00	—	—	—	—	—	—
b	—	0.54	0.58	1.00	0.77	1.00	0.69
c	—	0.46	0.42	—	0.23	—	—
d	—	—	—	—	—	—	0.31
Mdh-1							
n	16	12	10	5	56	11	15
a	—	—	—	0.30	0.02	0.05	—
b	1.00	1.00	1.00	0.70	0.96	0.91	1.00
c	—	—	—	—	0.02	0.04	—
Mdh-2							
n	16	12	10	5	56	11	14
a	1.00	1.00	0.85	1.00	1.00	1.00	1.00
b	—	—	0.15	—	—	—	—
Me							
n	14	12	13	4	56	11	16
a	1.00	—	—	—	—	—	—
b	—	0.46	0.42	0.62	0.54	0.46	0.47
c	—	0.54	0.58	0.38	0.46	0.54	0.53

Table 2

Continued.

Locus/ allele	*N. australis* 1000	*T. tanmurranus* 1003	*C. monodon* 1015	*C. primum* 1017	*C. primum* 1018	*C. primum* 1014	*C. grossum* 1011
Mpi							
n	16	12	13	4	56	11	13
a	—	—	0.04	0.50	0.56	0.55	0.15
b	1.00	1.00	0.96	0.50	0.44	0.45	0.85
Pep-1							
n	11	12	13	5	56	11	12
a	—	0.42	—	—	—	—	—
b	—	0.58	0.04	0.90	0.72	0.82	0.71
c	0.55	—	0.96	0.10	0.28	0.18	0.29
d	0.45	—	—	—	—	—	—
Pep-2							
n	11	12	13	5	55	11	16
a	0.41	0.17	0.08	—	0.07	—	0.28
b	0.59	0.83	0.88	1.00	0.88	0.95	0.72
c	—	—	0.04	—	0.05	0.05	—
Pep-3							
n	14	12	13	5	54	11	16
a	—	0.12	—	—	—	—	—
b	—	0.42	0.96	0.60	0.48	0.77	0.37
c	—	0.46	0.04	0.40	0.52	0.23	0.63
d	1.00	—	—	—	—	—	—
Pgm-1							
n	14	12	13	5	56	11	16
a	1.00	0.04	—	—	—	—	—
b	—	0.96	0.81	—	0.26	—	—
c	—	—	0.19	1.00	0.74	1.00	1.00
Pgm-2							
n	13	12	13	5	53	11	15
a	—	0.62	0.31	0.40	0.18	0.09	0.23
b	0.62	0.25	—	—	—	—	—
c	—	0.13	0.69	0.60	0.82	0.91	0.77
d	0.38	—	—	—	—	—	—
Pgd							
n	14	12	13	4	42	9	11
a	1.00	1.00	1.00	0.75	0.98	0.78	0.91
b	—	—	—	0.25	0.02	0.22	0.09
Sord							
n	16	12	13	5	53	11	13
a	0.03	0.08	—	—	—	—	0.08
b	0.97	0.92	—	—	0.01	—	0.08
c	—	—	0.85	1.00	0.99	1.00	0.84
d	—	—	0.15	—	—	—	—
$\bar{n}$	14.2	12.0	12.6	4.8	51.3	10.4	14.4
$\bar{A}$	1.2	1.7	1.7	1.6	1.9	1.7	1.6
P	0.19	0.48	0.62	0.52	0.71	0.62	0.57
$\bar{H}$	0.08	0.17	0.15	0.24	0.22	0.19	0.19

* $\bar{n}$, mean sample size per locus; $\bar{A}$, mean no. of alleles per locus; P, proportion of polymorphic loci (monomorphic loci: *G6pd, Got-2* and *Gapd* excluded from this table); $\bar{H}$, mean individual heterozygosity.

Table 3

Matrix of genetic distance coefficients for species of *Ningbingia, Turgenitubulus,* and *Cristilabrum*. Below diagonal: modified Rogers' distance. Above diagonal: Nei's unbiased genetic distance.

Population	1	2	3	4	5	6	7
1. *N. australis*	****	0.458	0.471	0.591	0.477	0.543	0.414
2. *T. tanmurranus*	0.569	****	0.212	0.314	0.264	0.310	0.257
3. *C. monodon*	0.577	0.402	****	0.199	0.145	0.151	0.168
4. *C. primum* 1017	0.626	0.476	0.399	****	0.016	0.000	0.051
5. *C. primum* 1018	0.571	0.432	0.334	0.167	****	0.013	0.026
6. *C. primum* 1014	0.607	0.470	0.349	0.132	0.129	****	0.039
7. *C. grossum*	0.545	0.433	0.363	0.238	0.157	0.198	****

to be very similar to *C. grossum* ($\bar{D}$ = 0.039, range: 0.026–0.051) but *C. monodon* was almost four times as well differentiated ($\bar{D}$ = 0.165, range: 0.015–0.20). In contrast, the intergeneric genetic distances were much greater:

Cristilabrum–Turgenitubulus $\bar{D}$ = 0.27 (0.21–0.31)
Cristilabrum–Ningbingia $\bar{D}$ = 0.50 (0.41–0.59)

These distance data were clustered using the UPGMA algorithm to produce a phenetic tree with a cophenetic correlation of 0.979 (Figure 2A). A phenogram based on modified Rogers' distances and the Distance Wagner procedure, with *N. australis* as the outgroup for purposes of rooting, was found after optimization to have a total length of 1.195 and a cophenetic correlation of 0.997 (Figure 2B).

DISCUSSION

In this first study of genetic variability in Australian camaenids, we have sought to establish whether these pulmonates are variable enough for their allozymes to be useful in population studies. Second, we needed to establish whether the morphologically defined species are sufficiently well differentiated from one another for allozymic variation to be used to construct phylogenetic trees. Studies of more than 20 loci in a large sample of *Cristilabrum primum* and relatives shows that both questions can be answered affirmatively. *Cristilabrum primum* is both variable and outcrossing (amphimictic) and the study of allozymic variation promises to lead to a phylogenetic hypothesis for the radiation as a whole. Although the samples representative of the other four species are small ($\bar{N}$ = 13.3) they are adequate to estimate both *P*, $\bar{H}$, and *D* as 21 loci were examined (Gorman & Renzi, 1979; Nei, 1987).

We have found that *Cristilabrum primum* (*P* = 0.62–0.71, $\bar{H}$ = 0.19–0.22) is more variable than average for mollusks; Nevo *et al.* (1984) summarized published data for 46 species finding $\bar{P}$ = 0.47 and $\bar{H}$ = 0.15. Other highly variable mollusks include *Crepidula onyx* (*P* = 0.56–0.70, $\bar{H}$ = 0.14–0.17) (Woodruff *et al.*, 1986), *Nautilus pompilus* (*P* = 0.50–0.66, $\bar{H}$ = 0.10–0.16) (Woodruff *et al.*, 1987), *Oncomelania hupensis* (*P* = 0.52–0.62, *H* = 0.19–0.21) (Woodruff *et al.*, 1988), and *Partula mooreana* (*P* = 0.74, $\bar{H}$ = 0.17) (Johnson *et al.*, 1986b). Similar high values characterize the other two species of *Cristilabrum* examined and *Turgenitubulus tanmurranus*. In contrast, *Ningbingia australis* (*P* = 0.19, $\bar{H}$ = 0.08) is less variable than the typical mollusk and only one-third as variable as the other Ningbing endemics. Although from Figure 2 it would be tempting to suggest that *Ningbingia* may have been derived from a variable *Cristilabrum-Turgenitubulus* ancestor and lost its variation during its origination, morphological evidence strongly contradicts a *Turgenitubulus* to *Ningbingia* transition. The former genus has a "dead end" specialized genitalic complex, which is readily derivable from the structures seen in *Cristilabrum,* but very different from the *Ningbingia* pattern (Solem, unpublished). It is also important to remember that intraclade levels of polymorphism can be highly variable. Even within a genus two- to three-fold differences in polymorphism are not unusual: *e.g.,* within the genera mentioned earlier in this paragraph, *Crepidula adunca* (*P* = 0.34), *Nautilus macromphalus* (*P* = 0.33), *Oncomelania quadrasi* (*P* = 0.20), and *Partula exigua* (*P* = 0.47) (above-cited references). Further discussion of the reduced levels of variation in *Ningbingia* will have to await comparative data on other species in the radiation.

Despite the high levels of variation within populations only minor differences were detected between congeneric species. The three species of *Cristilabrum* share most of the same alleles and differ primarily in allelic frequencies (Table 2). No single locus distinguishes *C. primum* from *C. grossum* and only half the specimens of the latter taxon possess species-specific alleles (Ldh^d and $Sord^a$). Similarly, allelic frequency differences at four loci contribute most to the distinction between *C. primum* and *C. monodon*; alleles specific to the latter taxon were detected at 1–2 loci in only half the individual snails. Representatives of the other genera were more differentiated but still shared 53% (*Turgenitubulus*) or 26% (*Ningbingia*) of their alleles with *Cristilabrum*. Nei's (1978) unbiased genetic distance (*D*) is particularly useful for quantifying such allelic frequency-dependent differences. As the calculated values of *D* are low to moderate (0–0.59), and as the number of loci ex-

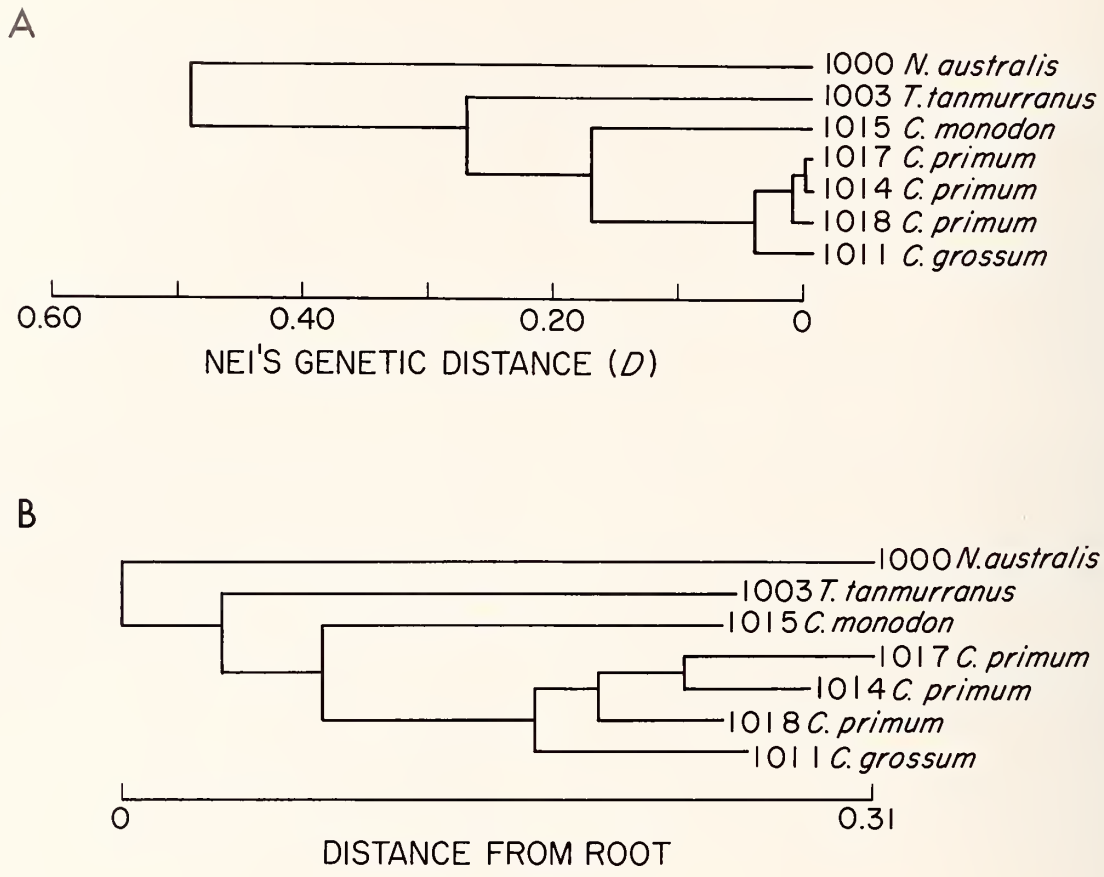

Figure 2

A. Phenetic tree based on variation at 21 loci. B. Wagner tree rooted with *Ningbingia australis* as the outgroup.

amined and the sample sizes are adequate (Nei, 1987), D should provide a reliable estimate of genetic differentiation in this group.

Although there is no simple linear relationship between Nei's D and level in a taxonomic hierarchy, some broad generalizations are possible. Within a clade D typically increases between populations, species, and genera. Thorpe's (1983) survey of over 7000 published conspecific comparisons showed that 98% of the intraspecific D estimates were ≤ 0.10. In contrast, he found that among 900 interspecific congeneric comparisons D was typically about 0.40 (range: 0.03–>1.00). Similar results were obtained by Woodruff *et al.* (1988) in their survey of 20 molluscan genera: genetic distances between conspecific populations were typically $D \leq 0.10$ and between congeneric species, $D > 0.05$ (typically 0.20–0.60).

In this light, the species of the Ningbing radiation are less well differentiated than might be expected. *Cristilabrum primum* and *C. grossum* ($D = 0.04$) are barely distinguishable at the 21 loci studied. *Cristilabrum monodon* is only moderately differentiated ($D = 0.17$) from its congeners. *Turgenitubulus* and *Ningbingia* differ from *Cristilabrum* at levels more typical of other animal species than of genera ($D = 0.27$–0.50). There was, however, no reason to suppose that these previously unstudied camaenid taxa would show "typical" levels of genetic divergence; one of the main aims of the present report is to establish their clade-specific pattern.

Within *Cristilabrum primum* our comparison of the three samples revealed no significant differentiation. Genetic distance values ranged from zero to 0.016 and at this level of variation the standard errors of D exceed the D-values themselves (Nei *et al.*, 1985). This genetic homogeneity conforms with a pattern of conchological and genital stability; only adult shell size shows significant variation. The shells of *C. primum* average 16.25–17.05 mm in diameter where their shelter sites are exposed to morning (east side) or midday sun (north side), but average 19.58–20.53 mm, or 20% larger, when the shelter site faces south and is shaded almost the entire day. No geographic differences in genital structure were observed.

Comparing *Cristilabrum primum* with its neighbor to the south, *C. grossum*, we estimated $\bar{D} = 0.039$ (0.026–0.051); values not significantly different from zero. One

Table 4

Summary of conchological and anatomical differences between three species of *Cristilabrum*.*

Character	*C. monodon* (n = 480)	*C. primum* (n = 1123)	*C. grossum* (n = 700)
Shell height (mm):	8.89 (7.4–10.5)	9.01 (7.2–12.75)	11.03 (9.05–13.4)
Shell diameter (mm):	17.37 (14.8–19.5)	17.29 (14.5–21.8)	20.72 (17.75–23.7)
No. of whorls:	5½ (5⅛–6)	4⅞+ (4⅜–5⅞+)	5⅜ (4⅞–6+)
Shell sculpture:	greatly reduced	very prominent above and below shell periphery	prominent above, reduced on base
Lip ridge on:	palatal wall	basocolumellar wall, recessed	basal wall
Palatal groove:	absent	weak	strong
Shell periphery:	rounded	sharply angled	weakly angled
Vagina/penis length	equal	equal	0.67
Free oviduct	very short	medium short	short
Penis apical plug	small	small	large
Penis stimulator	reduced	medium	large
Penis and sheath rel. length	1.3×	2×	4×
Penis sheath wall thickened	all	lower ⅓	lower ½

* Source: Solem, 1981, 1985, unpublished.

might conclude that the two taxa are really conspecific but this would be an error for several reasons. First, as mentioned above D values do not dictate taxonomic decisions. There are many other cases of very low interspecific genetic distances: for some sibling species of *Drosophila* $\bar{D} = 0.03$ (Prakash, 1969; Carson, 1982); for interspecific comparisons in many genera of birds $\bar{D} \leq 0.05$ (Corbin, 1987). Turning to land snails, Johnson *et al.* (1986a) found interspecific genetic distances between four species of *Samoana* were $D \leq 0.03$. In *Cerion,* three pairs of semispecies showed genetic distances of $\bar{D} = 0.05$ (Gould & Woodruff, 1978, 1986, 1987). Second, there is abundant evidence that *Cristilabrum primum* and *C. grossum* have acquired other attributes of good species. A number of shell and genital differences are listed in Table 4. It is highly significant that no structurally intermediate examples were seen among 2305 adult specimens examined for shell features. Differences in the absolute and relative sizes of terminal genital organs are equivalent to those seen in sympatric species pairs of *Cristilabrum* (see Solem, 1985) and thus indicate species-level differentiation. Although direct observational data are lacking, we hypothesize that the major differences in the genitalia play key roles in species isolation and recognition. Speciation thus appears to have involved significant changes in morphology but left the detectable genetic (allozymic) architecture unchanged. There is, of course, no reason to expect the evolution of morphological and behavioral traits to involve a concomitant differentiation at allozyme loci.

At the north end of its range, *Cristilabrum primum* is replaced by *C. monodon* (Figure 1C). Although similar in size and shape, they differ grossly in shell sculpture, whorl count, and penis-penis sheath structures (Table 4). They also are better differentiated allozymically. The genetic distance is $\bar{D} = 0.17$ and there is no evidence of introgression between the samples collected 200 m apart on the same hill. *Cristilabrum monodon* occupies the northwest corner of the main mass, but also occurs alone on the large outcrop to the north. It is most similar in both genital anatomy and shell features to *C. bubulum,* which lives on limestone masses to the southwest of the *monodon-primum-grossum* habitat.

In this preliminary report we demonstrate the results of two different methods of clustering these taxa based on allozyme variation. First, using a phenetic approach we can cluster taxa solely on the basis of their average genetic distances (Figure 2A). This approach allows for the importance of changes in allele frequencies. The phenogram will not reflect phylogenetic relationships, however, unless it is assumed that the rates of evolutionary change have been constant among the lineages. If the expected rate of gene substitution is approximately constant among these related snails, then the measure of divergence employed, Nei's D, will be directly proportional to evolutionary time. In that case the UPGMA phenogram will give correct phylogenetic topology and branch lengths. It would, however, be premature to regard Figure 2A as a phylogenetic tree at this stage of our investigation.

An alternative approach to the problem of phylogenetic reconstruction is the Distance Wagner method (Farris, 1972) which we have used here with a metric, the modified Rogers' distance (Wright, 1978). We studied mid-point rooted and out-group rooted trees and present one of the latter for discussion purposes (Figure 2B). It has been argued that if the taxa under consideration have a history of demographic bottlenecks then this algorithm should be preferred over the average distance method. Different branch lengths are presumed to reflect different rates of